

SENR Research with Distinction Program

**Consistent individual differences in boldness of an African cichlid fish
(*Pseudocrenilabrus multicolor*).**

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Abstract

Recent research in animal behavior acknowledges that in many species, individuals exhibit consistent differences in behavior across time and ecological contexts (known as behavioral syndromes or animal personalities). Individual variation in behavior can be described as a trait (bold, shy, curious, social, aggressive, etc.). The behavioral trait studied here is boldness, or how an animal reacts to a situation perceived as dangerous. Boldness was measured by observing an individual's foraging and anti-predation behavior after a simulated predation attack using a model predator. Individuals used in the study were F1 generation of wild caught cichlids (*Pseudocrenilabrus multicolor*) which came from two environmentally distinct habitats in Uganda, Africa (Bwera – swamp with low turbidity, and Bunoga – river with high turbidity). Preliminary results suggest individuals from the swamp population display individual differences in both foraging and anti-predation contexts, and their behavior is significantly repeatable. Data collection of the river habitat population is still being taken and further analysis is planned.

Introduction

In the last half decade there has been a dramatic increase in publication rate on non-human empirical studies on animal personality. There have been many studies, with considerable detail, in primates, domesticated animals, and laboratory rodents. Recently, behavioral ecologists have documented animal personalities in several 'non-model' organisms including mammals, birds, lizards, amphibians, molluscs, arthropods, and fish (Sih et al., 2004a). Research on fishes has contributed to the early documentation of individual consistency in behavior and continues to lead the way with new research avenues within the animal personality field (Conrad et al., 2011).

Changes in animal behavior often represent the first response for animals coping with changes to their environment. Recent studies have emphasized behavioral roles in applied ecological issues, like exotic invasions or species abilities to cope with human-induced rapid environmental change (Sih et al., 2012). It is very important, from a conservation point of view, to understand why and how a species continues to persist through habitat degradation from human-induced, rapid environmental change.

Animal personalities are often used synonymously with behavioral syndromes, which is a suite of correlated behaviors across multiple contexts (e.g. bold behavior in the contexts of foraging, mating, anti-predation, parental care, or dispersal) (Sih et al., 2004a). Behavioral syndromes in fishes has been described in five axes: (1) boldness – shyness, (2) exploration – avoidance, (3) activity, (4) aggressiveness, and (5) sociability (Conrad et al., 2011). Throughout this study I will be focusing only on the boldness – shyness axis of fish behavior. Boldness has been defined as a measure of an

individual's reaction to a situation perceived as dangerous, such as the presence of a predator.

Behavioral syndromes are extremely important to ecology because they imply limited plasticity in behavioral traits, thus constraining the ability of animals to behave in an optimal fashion across multiple situations; especially in the scope of rapid environmental change. For example, when boldness and aggressiveness are positively correlated in a population, selective pressures that may favor aggressiveness could result in bold behavioral types, when boldness is not favored (Conrad et al., 2011). Some consequences of this could be that individuals with a bold behavioral type may take unnecessary risks and suffer higher mortality in dangerous environments, or in low density situations aggressiveness can be a wasted effort (Sih et al., 2012). Boldness can also be a very rewarding benefit to an individual in the scope of nutrient acquisition (Sih et al., 2004b).

Hypothesis and Predictions

The hypothesis proposed for this project follows previous research and suggests that fish will display consistent individual behavior. Based on this hypothesis, I predict that some individuals will be bolder than others, and I also predict that fish from the river habitat population (Bunoga) will exhibit a higher level of boldness than the swamp habitat population (Bwera). The latter prediction is based on the fact that the parent Bunoga population encounters high turbidity frequently, which reduces visibility in the water column. The reduction in visibility may reduce visual predation cues in their habitat, allowing the population to display increasingly bold behavior.

Objectives

The main objectives of this study are to: (1) determine if F1 generation offspring from wild-caught adult populations in Uganda, Africa experiencing human-induced environmental change will exhibit consistent individual behavior across multiple contexts, and (2) determine if the two populations from environmentally distinct habitats produce offspring that are behaviorally different. These objectives will be met by: (a) rearing eight broods of fish, originating from two different environmentally distinct habitats, (b) designing and building an experimental setup to test the behavior of *P. multicolor*, and (c) determining if variation in the tested behavior is statistically significant.

Methods

All parents and offspring are housed in the Kottman Hall Greenhouse located at 2021 Coffey Rd. Columbus, Ohio, under IACUS Protocol #2014A0000005. All adults, from which the test subjects (F1 offspring) are generated, were collected by Dr. Gray during fieldwork in Uganda, Africa in July 2014.

Parent populations and offspring: Eight broods (4x broods per population) of offspring will be generated through natural breeding of wild-caught adults (*P. multicolor*) originating from two environmentally distinct habitats in Uganda, Africa. The first population, Bwera, is a swamp habitat with relatively low dissolved oxygen and turbidity, and low environmental variation (Crispo and Chapman 2008). The second population, Bunoga, is a river habitat which suffers from human-induced environmental degradation with high dissolved oxygen and turbidity, and high environmental variation (Crispo and

Chapman 2008). Each brood will be housed in an isolated tank and fed fry food for approximately two months. Fish are then weaned onto the adult food (crushed “tropical crisps”, Tetramin) for an additional month. After fish reach 3 months in age, 5 individuals are randomly selected from each brood and placed into individual experimental tanks. Once placed in the experimental tanks fish will be allowed to acclimate to the new tank for one week before the initiation of trials.

Experimental setup and design: Experimental tanks (20x 5-gallon tanks) are arranged with identical filtration and areas of refuge. Weekly water changes will be performed to ensure high water quality. Each tank will be split into thirds, with the first third containing the filtration sponge and fake foliage for refuge. The second and third portions of the tank will be open and only separated by a clear barrier during the pre-trial protocol. Feeding cycles will be identical and only change when a fish is selected for a trial, during which food is withheld for 24 hours to allow the fish to enter a post-absorptive state to standardize hunger drive. Before each behavioral trial, water quality parameters are recorded (dissolved oxygen, conductivity, temperature, pH, turbidity, ammonia, and nitrite) to ensure similar environments across tanks. The total experiment will consist of 120 trials (3 trials/fish x 5 fish/brood x 4 broods/population x 2 populations). Fish are lured out of refuge with food that is placed on the opposite side of the clear barrier (i.e. fish can see but not eat food because of barrier), and immediately thereafter a model predator (or other stimulus such as a potential mate) is introduced to scare the fish back into the refuge. Once the fish is back in refuge, the predator is removed after approximately 5-10 seconds, and then the clear barrier is removed. Once both predator and barrier is removed the 5-minute timed behavioral trial begins.

Preliminary Statistics: Each trial is videotaped for later analysis in the lab. From each video trial we quantify the number of food pecks, latency to leave refuge (initially), and proportion of time spent in refuge. These response variables were chosen because of the similarity to other scholarly studies (Wright et al., 2006; Faser et al., 2001; Harcourt et al., 2009; Toms et al., 2010). Initial results for one population (Bwera) have been obtained using SPSS statistical software. Data will be analyzed using ANOVA univariate analysis, then the mean square results to calculate repeatability (fraction of individual variation due to differences between individuals) (Bell et al., 2009).

Preliminary Results

Fish raised from the Bwera population exhibited individual differences in behavior in the foraging (food pecks: $F_{19,40} = 2.235$, $p = 0.016$) context and one anti-predation (proportion of time spent in refuge: $F_{19,40} = 2.126$, $p = 0.022$) context; however, there was no statistical significance (latency to leave refuge: $F_{19,40} = 1.469$, $p = 0.151$) in the other anti-predation context. The Bwera population also exhibited significant repeatability (Figure 1) in the foraging (food pecks: $r = 0.292$, ± 0.149) context and one anti-predation (proportion of time spent in refuge: $r = 0.277$, ± 0.149) context. Again, there was no significant repeatability in the other anti-predation context (latency to leave refuge: $r = 0.135$, ± 0.1455). Only a small number of fish raised from the Bunoga population have been tested to date. A preliminary analysis suggests no signs of individual behavior ($p = 0.350$, 0.630 , and 0.511), or repeatability ($r = -0.1495 \pm 0.208$, 0.0775 ± 0.275 , and -0.0341 ± 0.249); this is likely a result from the very small sample size and is subject to change as the remaining trials are performed and analyzed.

Discussion

Only limited speculations of the predictions can be made with the current results, as only half of the experiment is complete; however, there is evidence for consistent individual behavior in some foraging and anti-predation contexts in the Bwera population, and I suspect that the Bunoga population may potentially exhibit consistent individual behavior as well. Since all specimens are being preserved, there is potential that physiological aspects of each fish can help aid in the understanding of bold behavior; for example, in a study of a tropical poeciliid, researchers suggested that boldness was positively correlated to standard length (Brown et al., 2005).

Some future directions of this research could be developed to help explain how boldness can change in relation to ontogeny, how rearing fish under different environmental conditions (e. g. high vs. low turbidity, or high vs. low dissolved oxygen) can affect boldness, and/or how boldness changes in relation to different introduced stimuli (boldness in relation to presence of mate and/or competitor). By answering these questions, we will gain a better understanding of the link between behavior, environmental change, and biodiversity.

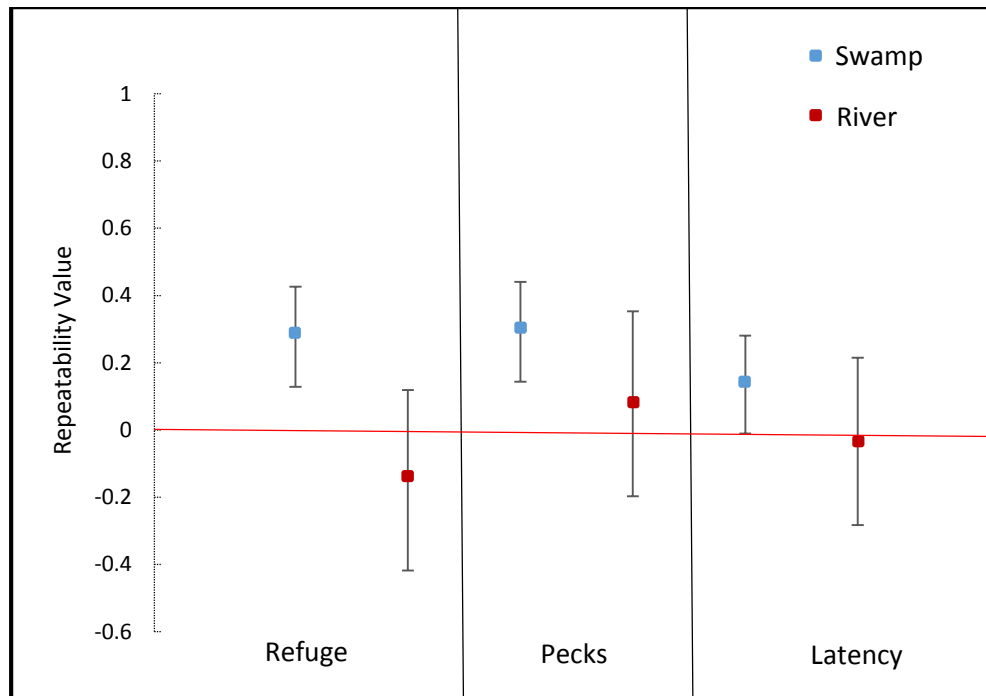


Figure 1. Repeatability scores of Bwera (swamp) and Bunoga (river) populations for foraging and anti-predation. Left section: proportion of time spent in refuge. Middle section: number of pecks at food. Right section: Latency to emerge from refuge.

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